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Characterization of bone mineral density in male-to-female transsexuals receiving treatment for reassignment surgery: 15 years of follow-up

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Abstract

Background: Cross-sex hormone treatment in male-to-female (M2F) transsexuals appears reasonably safe. Little is known about its long-term use. The aim of our study was to evaluate the effect of long-term high dose estrogens, plus the antiandrogen cyproterone acetate, on bone composition and on biochemical/hormonal parameters in M2F transsexuals.

Methods: A retrospective analysis was performed on 45 young M2Fs (mean age 39.5 years; body mass index (BMI) = 22) receiving estrogens (previously 100 μg ethinyl estradiol, now 2–4 mg oral estradiol valerate/day or 100 μg transdermal estradiol/day) plus the antiandrogen cyproterone acetate 100 mg/day. Data were retrieved from 20 subjects after reassignment surgery (mean hormonal treatment duration 15.6 years). A complete hormonal and biochemical assessment, as well as bone biochemical markers (parathyroid hormone (PTH), calcium, phosphorus, alkaline phosphatase and plasma pyridinoline cross-links), were evaluated. Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DEXA).

Results: All subjects had suppressed serum testosterone levels (mean = 0.57 nmol/l), whereas serum estradiol levels were within the supraphysiological range (mean = 880 pmol/l). A mild osteopenia at both lumbar spine and femoral neck was observed in 15 out of the 20 (75%) M2Fs (BMD = 0.89 ± 0.14 (mean $\pm$ standard deviation (SD)) g/cm^2 versus 1.1 ± 0.09, $p < 0.001$; lumbar T-score = -1.39 ± 0.84 versus 0.5 ± 1.10, $p < 0.0005$; femoral T-score = -1.12 ± 0.76 versus 0.08 ± 1.00, $p < 0.05$, respectively). No differences in plasma crosslink levels or in hormonal and biochemical parameters were found between subjects.

Conclusions: Our results indicate that cross-sex hormone treatment of M2Fs, independently of serum testosterone levels, seems acceptably safe over a median treatment period of 15 years in a consistent population of subjects. A protective role for estrogens on bone seems to be present in a minority of subjects.

Introduction

Osteoporosis-related fractures constitute a major public health concern in older men and women, in whom the lifetime fracture risk at age 45 has been estimated to be 24% [1]. Fracture risk is dependent on several extra and intra-skeletal factors, including muscle strength, bone mineral density (BMD), and bone geometry [2]. The maximal bone mass attained in life, the peak bone mass (PBM), is primarily dependent on genetic factors, but environmental factors also play a part. Sex steroids are important for both skeletal growth, PBM and bone mass maintenance in both the male and female skeleton [3–7]. More recently, studies [8] have strongly suggested that estradiol (E_2) plays a pivotal role, not only in female skeletal homeostasis but also in that of the male. Interestingly, changes in levels of estrogen or of the estrogen receptor (ER), and aromatase gene mutations have all been linked to significant skeletal alterations in males [9–18]. Indeed, due to a disruptive homozygous estrogen receptor-α mutation, one young man was found to be insensitive to the action of estrogen, leading to severe osteopenia, unfused epiphyses (resulting in linear growth into adulthood), increased gonadotrophin levels and evidence of premature atherosclerosis and endothelial dysfunction [15,18].

It is clear that while the role of E_2 in regulating bone metabolism in women is well established, the relevance of testosterone in regulating bone homeostasis in men is not fully understood. The traditional belief has been that testosterone is the major sex steroid regulating bone metabolism in men. In elderly men, however, a significant relationship has been found between E_2 serum levels and BMD: E_2 was shown to decrease bone resorption leading to increased bone mass, while testosterone together with E_2 appears to stimulate bone formation in men [11]. Testosterone can exert its biological effects either directly, by binding the androgen receptor (AR), or indirectly, by androgen aromatization to estrogen through estrogen receptor-α and/or -β (ER α/β) mediated effects [12]. Sex steroid receptors (ARKO/ERKO) are expressed both in growth plate cartilage and in bone. Interestingly, null sex steroid receptor transgenic mouse models indicate that these receptors mediate site-specific skeletal effects of sex steroids [13–15]. Although AR and ERs are present in the osteoblasts of both sexes, cortical bone contains more ARs than trabecular bone, providing a possible explanation for the higher cortical bone mass observed in men compared to women [16]. Additionally, the effects of androgens, compared to estrogens, are similar on trabecular bone but opposite on the cortical bone in males [17], suggesting that switching to cross-gender hormones in adulthood would lead to differential specific changes in bone density depending on the skeletal site.

Gender identity disorder, gender dysphoria, transgenderism and transsexualism are terms used to describe an individual's wish to live and to be accepted as a member of the opposite sex. The condition manifests as discomfort with one's phenotypic sex and a wish to have treatment to make one's body correspond to that of the opposite sex. In transsexualism, genotype and somatic differentiation do not conform to brain programming as male or female [19]. In transsexual individuals, the medical management of cross-gender reassignment is started and maintained using estrogen/antiandrogen treatment in male-to-female (M2F) transsexuals and androgen treatment in female-to-male (F2M) transsexuals [20].

While many studies have addressed the psychological problems only few, rather conflicting, studies have addressed the question of whether, in transsexuals, skeletal health is preserved during cross-sex hormone treatment. In fact, during the process of adaptation to the opposite sex, transsexuals receive cross-sex hormone treatment and thereby undergo a complete change in their hormonal milieu (estrogens in males and androgen in females). After reassignment surgery, which includes gonadectomy, hormone therapy must be continued. An unresolved question is whether, in the long term, all of the functions of the sex steroids of a subject are adequately covered by cross-sex hormone treatment and whether the administration of cross-sex hormones is appropriately safe [21]. The feminizing endocrine treatment in M2Fs is estrogen therapy in doses 2–3 times higher than the recommended doses for hormone replacement therapy (HRT) in postmenopausal women [22,23]. Additionally, to potentiate the effects of E_2 treatment, hormonal modulators or anti-androgens are used to further lower serum levels of testosterone or antagonize ARs and limit masculine sexual

characteristics. To this end, the most widely used drug is cyproterone acetate (usually 50 mg twice daily), a progestational compound with antiandrogenic properties [22–24].

Thus, the aim of our study was to evaluate whether long-term massive estrogen exposure, in the absence of testosterone, might affect the skeletal health of M2F transsexuals after their reassignment surgery.

Materials and methods

This study is based on a retrospective analysis of M2Fs treated with estrogens (previously 100 μg ethinyl estradiol, now 2–4 mg oral estradiol valerate/day or 100 μg transdermal estradiol/day) plus the antiandrogen cyproterone acetate 100 mg/day [21]. The study population included 45 healthy subjects with a mean age of 39.5 years (±9.1 standard deviation (SD)) and a body mass index (BMI) = 22 kg/m^2 (22.0 ± 2.9). Informed consent was obtained from all patients. Exclusion criteria were: previous treatment with oral antiandrogen cyproterone acetate and conjugated estrogens at the time of the first visit; history of use of gonadotropin releasing hormone (GnRH) agonists or any medications known to affect calcium metabolism (e.g. glucocorticoids, anticonvulsants, calcium and/or vitamin-D supplements, bisphosphonates or calcitonin); previous history of thrombosis or other vascular diseases. Overall, 20 men were included in this retrospective analysis and their hormonal and biochemical characteristics at the time of observation are reported in Table 1. All patients were interviewed regarding their medical history and sexual behaviour. All patients underwent a clinical examination, biochemical investigations including hormonal profile (glycemia, low density lipoprotein (LDL) and high density lipoprotein (HDL), cholesterol, tryglicerides, aspartate transaminase, glutamate transaminase, calcium, phosphates, alkaline phosphatase (ALP), pyridinium crosslinks (DPD), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), estradiol (E_2), testosterone, parathyroid hormone (PTH)). Bone mineral density (BMD) was evaluated using dual-energy X-ray absorptiometry (DEXA: Hologic QDR-1000) at the

Table 1 Complete hormonal, biochemical and bone markers in 20 M2F transsexuals at follow-up a mean of 15.6 years after reassignment surgery

	FSH	LH	T	E2	PTH	PRL	TSH	FT3	FT4	DPD	
M2F -O	60.6 ± 40	26.3 ± 19	0.27 ± 0.1	32.2 ± 27	23.8 ± 14	14.8 ± 8.5	2.31 ± 1.3	2.82 ± 0.7	1.05 ± 0.5	4048 ± 1331	n.s.s
M2F -NO	51.3 ± 35	21.1 ± 15	0.22 ± 0.1	47.3 ± 30	27.7 ± 6.8	11.2 ± 8.2	2.31 ± 1.5	2.78 ± 0.3	0.86 ± 0.1	3642 ± 1033	n.s.s
	GLY	**TOT COL**	**LDL COL**	**HDL COL**	**TRG**	**GOT**	**GPT**	**Ca**	**P**	**ALP**	
M2F-O	89.9 ± 5.4	182 ± 22	110 ± 26	59 ± 14	63 ± 26	35 ± 9	38 ± 16	9.1 ± 0.4	3.7 ± 0.7	52.6 ± 22.7	n.s.s
M2F -NO	91.5 ± 9.8	179 ± 25	104 ± 22	48 ± 11	50 ± 22	31 ± 13	35 ± 12	9.2 ± 0.3	3.4 ± 0.4	47.5 ± 14.9	n.s.s

M2F, male to female; M2F-O, M2F osteopenic; M2F-NO, M2F not-osteopenic; FSH, follicle stimulating hormone; LH, luteinizing hormone; T, testosterone; E2, eastradiol; PTH, parathyroid hormone; PRL, prolactin; TSH, thyroid secreting hormone; FT3, free triiodothyronine; FT4, free thyroxine; DPD, pyridinium crosslinks; Gly, glycemia; TOT COL, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TRG, tryglicerides; GOT, glutamic oxaloacetic aminotransferase; GPT, glutamic piruvic aminotransferase; P, phosphorus; ALP, alkaline phosphatase.

femoral neck and lumbar sites (L2–L4). The accepted female, instead of male, values were used for the evaluation of T scores and for the definition of osteopenia/osteoporosis due to the new hormonal milieu of the patients. Testosterone level was measured by radioimmunoassay (RIA) using a commercial kit (Diagnostic System Laboratories, Webster, TX, USA). Estradiol (E_2) was measured using chemiluminescent enzyme immunoassays (Architect Systems, Abbotts diagnostics, Germany), FSH, LH, and PRL were measured using direct chemiluminescence (ADVIA Centaur, Bayer Co, Germany) and PTH using an immunoradiometric assay kit (DiaSorin, Stillwater, MN, USA). Serum N-terminal telopeptide of procollagen type-I (NTx) was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Ostex Intern, Seattle, WA, USA).

Statistical differences were evaluated using the Student's t test and differences were considered significant when a p value of <0.05 was obtained.

Results

To evaluate whether anti-androgen therapy was adequate, mean serum testosterone levels were measured. As expected, serum testosterone levels were below the normal range for males (mean = 0.57 nmol/l). Additionally, E_2 levels were measured, and were above the supraphysiological range (mean = 880 pmol/l). Biochemical parameters were within normal ranges in all individuals, whereas hormonal values were similar to those that would be expected due to HRT (see Table 1). A significantly low BMD at both the lumbar spine and femoral neck was observed in 15 out of the 20 (75%) M2F subjects (BMD for osteopenic subjects = 0.89 ± 0.14 (mean $\pm$ SD) g/cm^2 versus 1.1 ± 0.09, $p < 0.001$; lumbar T score = -1.39 ± 0.84 versus 0.5 ± 1.10, $p < 0.0005$; femoral T score = -1.12 ± 0.76 versus -0.08 ± 1.00, $p < 0.05$, for osteopenic versus non-osteopenic subjects, respectively) (Fig. 1). Interestingly, serum levels of plasmatic crosslinks were within the normal range, indicating the presence of a normal bone remodeling process and, thus, strongly suggesting that the observed BMD alterations were likely to be the effect of previous hormonal modifications.

Discussion

The results described herein demonstrate that long-term treatment (average 15.6 years) with estrogens plus anti-androgens for M2F cross-sex treatment might contribute to the induction of significant alterations in BMD at both

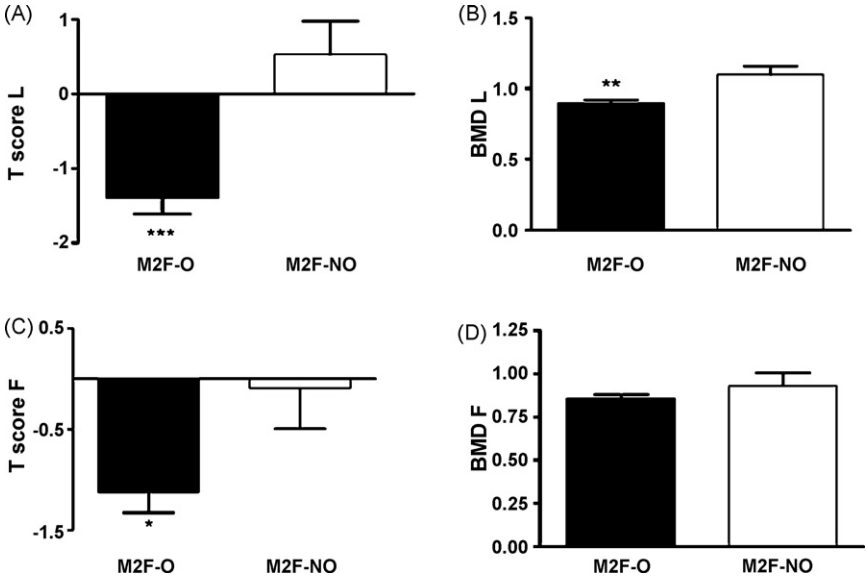

Figure 1 The graphs show the skeletal modifications at the level of both the lumbar spine and the femoral neck in 20 male-to-female (M2F) transsexuals. T-score values and bone mineral density (BMD) expressed as g/cm^2 are depicted for the lumbar spine (A and B, respectively) and femoral neck (C and D, respectively). M2F-O, M2F osteopenic; M2F-NO, M2F non-osteopenic.

the femoral neck and lumbar spine. Estrogen dosages used by this population were 2–3 times higher than the recommended doses for HRT in postmenopausal women, in whom the decrease in estrogen levels is strongly associated with decreased BMD and increased risk of fragility fractures.

The traditional belief has been that testosterone is the dominant sex hormone regulating bone metabolism in men. Interestingly, however, epidemiological studies have found no significant association, or even a negative association, between testosterone levels and BMD or vertebral fractures in aging men [25]. Data from Turner et al [26] showed that supraphysiologic testosterone therapy increased BMD at the hip while maintaining BMD at the spine in F2M transsexuals, leading to the speculation that the effects of testosterone may be the result of either its direct action on the bone or its indirect action through its aromatization to estrogens. Lorentzon et al. [27] reported that estradiol is a negative, whereas free testosterone is a positive, predictor of cortical bone size in young men at the age of peak bone mass. However, no data on the effects of estrogens in men were provided.

With regard to skeletal metabolism, there are few studies of M2F transsexuals receiving combinations of ethinyl estradiol and cyproterone acetate. Schlatterer et al. [28] reported a low risk of developing osteoporosis in transsexual individuals. However, the observed population of that study was only 10 patients. Interestingly, Lips et al. [29] have reported that continued estrogen treatment in orchidectomized M2Fs was not associated with bone loss, as was also demonstrated more recently by Van Kesteren et al., who observed that bone mass in both M2F and F2M transsexuals was not decreased after 1 year of cross-sex hormone administration [30]. Van Kesteren et al. also demonstrated, in 20 M2Fs, an increase in lumbar spine BMD after 1 year and a subsequent decrease after 28–63 months of cross-sex hormone treatment using cyproterone acetate (100 mg/day) in combination with ethinyl estradiol (100 mg/day) until sex reassignment surgery after 12–18 months. Bone turnover markers were significantly lower after 1 year of hormone treatment, demonstrating a decrease in bone turnover. There was no severe loss of lumbar BMD as long as the gonadotrophin levels were suppressed by adequate E_2 substitution [31]. Additionally, recent work by Haraldsen et al. [32] has shown that sex hormone treatment in patients with gender identity disorders induced minimal effects on BMD. However, the analysis was performed after a short period of treatment (at months 3 and 12), which cannot be considered a long enough period to appreciate the positive or negative effect of any drug on BMD.

Interestingly, despite it being known that suppression of testosterone production is more effective using GnRH than using the combination of ethinyl estradiol plus cyproterone acetate, we found no differences in testosterone suppression in our population of M2F transsexuals, thus suggesting that the reduced lumbar and femoral neck BMD found in our male-gender patients was possibly due to either the presence of excess estrogens or the complete lack of testosterone. This finding is in contrast with most studies carried out in M2F transsexuals, which have shown that androgen suppression over a period of 2–4 years preserves men from osteopenia mainly due to the known protective effects of estrogens on bone. Moreover, no significant differences between bone formation/resorption markers, as well as no difference in thyroid hormones, glucose, liver enzymes or lipid profile were found in the two groups of patients (osteopenic and nonosteopenic), suggesting that no important adverse event was determined by the ongoing estrogen therapy during the 15 years of follow-up. However, it is known that estrogens are able to regulate bone resorption by enhancing bone mass, while testosterone, together with estrogens, is responsible for bone formation in men. In men, homozygous mutations in the ERα gene or homozygous mutations in the aromatase gene have been found to be associated with osteopenia, unfused epiphyses and elevated indices of bone turnover. In men with homozygous mutations in the aromatase gene, E_2 treatment was able to reverse these circumstances successfully. All of this evidence suggests that it is possible that E_2 also plays a role in bone formation processes and in the acquisition of peak bone mass in men.

We are aware that our study lacks longitudinal evaluations and that data obtained in these patients may have been biased by factors other than hormonal treatments for reassignment surgery (e.g. cigarette smoking, drug

abuse, body weight alterations, etc). Moreover, in the present study it is not possible to assess any possible causal relationship between BMD changes and variation in circulating hormone levels. However, we can speculate that a different genetic susceptibility may be present in the male population and, in the presence of prolonged estrogen plus antiandrogen exposure, an increase in subcutaneous fat might contribute to a modification of the pro-inflammatory state, which could influence skeletal homeostasis over a long-term period. Indeed, in our subjects, only 25% had normal BMD, the remaining 75% were osteopenic.

In conclusion, the results of our study suggest that supraphysiological doses of estrogens plus anti-androgens in young M2F transsexuals might play a weak protective role in maintaining BMD. However, the relevance of either estrogens or testosterone in regulating bone turnover in men remains unclear, while the role of estrogens in regulating bone metabolism in women has been clarified more fully. We recommend that M2F transsexuals should be strictly monitored for BMD and bone remodeling markers, as well as hormonal levels, in order to evaluate the adequacy of HRT as well as possible bone loss over time. Further studies are warranted to fully clarify the potential positive/negative effects of sex steroid therapy on skeletal health in individuals with gender disorders.

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